File

CULTURE OF ANIMAL CELLS

A Manual of Basic Technique

R. Ian Freshney

Address all Inquiries to the Publisher
Alan R. Liss, Inc., 150 Fifth Avenue, New York, NY 10011

Printed in the United States of America

Second Printing, April 1984
Third Printing, November 1984
Fourth Printing, November 1986

Library of Congress Cataloging in Publication Data

Freshney, R. Ian.
Culture of animal cells.

Bibliography: p.
Includes index.
1. Tissue culture. 2. Cell culture. I. Title.
QH585.F74 1983 591'.07'24 82-24960
ISBN 0-8451-0223-0

Other areas of major interest include the study of cell interactions and intracellular control mechanisms in cell differentiation and development [Auerbach and Grobstein, 1958; Cox, 1974; Finbow and Pitts, 1981] and attempts to analyze nervous function [Bornstein and Murray, 1958; Minna et al., 1972]. Progress in neurological research has, however, not had the benefit of working with propagated cell lines as propagation of neurons has not so far been possible *in vitro* without resorting to the use of transformed cells (see Chapter 20).

Tissue culture technology has also been adopted into many routine applications in medicine and industry. Chromosomal analysis of cells derived from the womb by amniocentesis can reveal genetic disorders in the unborn child, viral infections may be assayed qualitatively and quantitatively on monolayers of appropriate host cells, and the toxic effects of pharmaceutical compounds and potential environmental pollutants can be measured in colony-forming assays.

Further developments in the application of tissue culture to medical problems may follow from the demonstration that cultures of epidermal cells form functionally differentiated sheets in culture [Green et al., 1979], and endothelial cells may form capillaries [Folkman and Haudenschild, 1980], suggesting possibilities in homografting and reconstructive surgery using an individual's own cells. The introduction of heterologous genetic material into mammalian cells [Willecke et al., 1979; Wigler et al., 1979], although somewhat overshadowed by current propagation in bacteria, may yet prove a desirable means for producing biologically significant compounds such as growth hormone and insulin. Similarly, the production of monoclonal antibodies [Kohler and Milstein, 1975] in hybrids between human plasma cells and human myeloma cells may prove a valuable technique for the production of specific antibodies.

It is clear that the study of cellular activity in tissue culture may have many advantages; but in summarizing these, below, considerable emphasis must also be placed on its limitations, in order to maintain some sense of perspective.

ADVANTAGES OF TISSUE CULTURE

Control of the Environment

The two major advantages, as implied above, are the control of the physicochemical environment (pH, temperature, osmotic pressure, O_2, CO_2 tension), which may be controlled very precisely, and the physiological conditions, which may be kept relatively constant but cannot always be defined. Most media still require supplementation with serum which is highly variable [Olmsted, 1967; Honn et al., 1975], and contains undefined elements such as hormones and other regulatory substances. Gradually, however, the functions of serum are being understood; and as a result, it is being replaced by defined constituents [Birch and Pirt, 1971; Ham and McKeehan, 1978; Barnes and Sato, 1980].

Characterization and Homogeneity of Sample

Tissue samples are invariably heterogeneous. Replicates even from one tissue vary in their constituent cell types. After one or two passages, cultured cell lines assume a homogeneous, or at least uniform, constitution as the cells are randomly mixed at each transfer and the selective pressure of the culture conditions tends to produce a homogeneous culture of the most vigorous cell type. Hence, at each subculture each replicate sample will be identical, and the characteristics of the line may be perpetuated over several generations. Since experimental replicates are virtually identical, the need for statistical analysis of variance is seldom required.

Economy

Cultures may be exposed directly to a reagent at a lower and defined concentration, and with direct access to the cell. Consequently, less is required than for injection *in vivo* where $>90\%$ is lost by excretion and distribution to tissues other than those under study.

DISADVANTAGES

Expertise

Culture techniques must be carried out under strict aseptic conditions, because animal cells grow much less rapidly than many of the common contaminants such as bacteria, molds, and yeasts. Furthermore, unlike microorganisms, cells from multicellular animals do not exist in isolation, and consequently, are not able to sustain independent existence without the provision of a complex environment, simulating blood plasma or interstitial fluid. This implies a level of skill and understanding to appreciate the requirements of the system and to diagnose problems as they arise. Tissue culture should not be undertaken casually to run one or two experiments.

Quantity

A major limitation of cell culture is the expenditure of effort and materials that goes into the production of relatively little tissue. A realistic maximum per batch for most small laboratories (2 or 3 people doing tissue culture) might be 1–10 g of cells. With a little more effort and the facilities of a larger laboratory, 10–100 g is possible; above 100 g implies industrial pilot plant scale, beyond the reach of most laboratories, but not impossible if special facilities are provided.

The cost of producing cells in culture is about ten times that of using animal tissue. Consequently, if large amounts of tissue (>10 g) are required, the reasons for providing them by tissue culture must be very compelling. For smaller amounts of tissue ($\leqslant 10$ g), the costs are more readily absorbed into routine expenditure; but it is always worth considering whether assays or preparative procedures can be scaled down. Semimicro- or micro-scale assays can often be quicker due to reduced manipulation times, volumes, centrifuge times, etc. and are often more readily automated (see under Microtitration, Chapter 19).

Instability

This is a major problem with many continuous cell lines resulting from their unstable aneuploid chromosomal constitution. Even with short-term cultures, although they may be genetically stable, the heterogeneity of the cell population, with regard to cell growth rate, can produce variability from one passage to the next. This will be dealt with in more detail in Chapters 12 and 18.

MAJOR DIFFERENCES *IN VITRO*

Many of the differences in cell behavior between cultured cells and their counterparts *in vivo* stem from the dissociation of cells from a three-dimensional geometry and their propagation on a two-dimensional substrate. Specific cell interactions characteristic of the histology of the tissue are lost, and, as the cells spread out, become mobile and, in many cases, start to proliferate, the growth fraction of the cell population increases. When a cell line forms it may represent only one or two cell types and many heterotypic interactions are lost.

The culture environment also lacks the several systemic components involved in homeostatic regulation *in vivo*, principally those of the nervous and endocrine systems. Without this control, cellular metabolism may be more constant *in vitro* than *in vivo*, but may not be truly representative of the tissue from which the cells were derived. Recognition of this fact has led to the inclusion of a number of different hormones in culture media (see Chapter 9) and it seems likely that this trend will continue.

Energy metabolism *in vitro* occurs largely by glycolysis, and although the citric acid cycle is still functional it plays a lesser role.

It is not difficult to find many more differences between the environmental conditions of a cell *in vitro* and *in vivo* and this has often led to tissue culture being regarded in a rather skeptical light. Although the existence of such differences cannot be denied, it must be emphasized that many specialized functions are expressed in culture and as long as the limits of the model are appreciated, it can become a very valuable tool.

Origin of Cells

If differentiated properties are lost, for whatever reason, it is difficult to relate the cultured cells to functional cells in the tissue from which they were derived. Stable markers are required for characterization (see Chapter 15); and in addition, the culture conditions may need to be modified so that these markers are expressed (see next chapter).

DEFINITIONS

There are three main methods of initiating a culture [Schaeffer, 1979] (see Glossary and Fig. 1.2): (1) *Organ culture* implies that the architecture characteristic of the tissue *in vivo* is retained, at least in part, in the culture. Toward this end, the tissue is cultured at the liquid/gas interface (on a raft, grid, or gel) which favors retention of a spherical or three-dimensional shape. (2) In *primary explant culture* a fragment of tissue is placed at a glass (or plastic)/liquid interface where, following attachment, migration is promoted in the plane of the solid substrate. (3) *Cell culture* implies that the tissue or outgrowth from the primary explant is dispersed (mechanically or enzymatically) into a cell suspension which may then be cultured as an adherent monolayer on a solid substrate, or as a suspension in the culture medium.

Organ cultures, because of the retention of cell interactions as found in the tissue from which the culture was derived, tend to retain the differentiated properties of that tissue. They do not grow rapidly (cell proliferation is limited to the periphery of the explant and is restricted mainly to embryonic tissue) and hence cannot